

Final Report

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**Mathematical Models of Continuous
Flow Electrophoresis
Electrophoresis Technology**

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ABSTRACT

This work focused on two aspect of continuous flow electrophoresis:

- (i) The structure of the flow field in continuous flow devices;
- (ii) The electrokinetic properties of suspended particles relevant to electrophoretic separations.

Mathematical models were developed to describe flow structure and stability, with particular emphasis on effects due to buoyancy. To describe the fractionation of an arbitrary particulate sample by continuous flow electrophoresis a general mathematical model was constructed. In this model, chamber dimensions, field strength, buffer composition, and other design variables can be altered at will to study their effects on resolution and throughput. All these mathematical models were implemented on a digital computer and the codes are available for general use. The results provide a rationale for microgravity experimentation and the computer models can be used to establish the ultimate resolving power of devices in terrestrial or microgravity environments.

Experimental and theoretical work with particulate samples probed how particle mobility is related to buffer composition. It was found that ions on the surfaces of small particles are mobile, contrary to the widely accepted view. This influences particle mobility and suspension conductivity. A novel technique was used to measure the mobility of particles in concentrated suspensions. This showed that particle-particle interactions tend to cancel and particles retain the mobility measured at infinite dilution. Thus, intrinsic mobility differences will not be degraded by particle-particle interactions, per se. Measurements of suspension conductivity disclosed a strong effect due to the presence of the particles.

The results have been reported to the scientific community as follows: 13 papers in the refereed literature, (4 other papers are still in preparation); 22 papers at technical meetings; 1 PhD thesis; 1 MSE Thesis; and 4 BSE theses.

As a result of this research and complementary work at MSFC our understanding of the basic processes has advanced to a point where a definitive set of experiments can be designed to establish the resolving power and capacity of continuous flow devices with particulate samples.

SUMMARY & CONCLUSIONS

This report is a description of work done at Princeton on continuous flow electrophoresis(CFE) of particulate samples. Since the publications and theses from this work run to several hundred pages they have not been included; copies are available on request from the Principal Investigator.

The program began with the objective of developing mathematical models of the device. Later the study was expanded to include the electrokinetic properties of suspended particles displaying some of the properties of samples that would be separated in a CFE device. The principal findings are as follows:

1. Wide-gap machines are particularly susceptible to buoyancy driven instabilities resulting from any adverse temperature gradient (i.e. anti-parallel to the gravity vector). Such instabilities lead to non-rectilinear flows which distort the sample stream. The adverse gradient may arise in the entrance region of downflow machines, where the buffer flow first encounters the electric field and heating begins, or from uneven heating and cooling of the chamber boundaries in upflow or downflow configurations. Much of the adverse effect could be overcome by careful control of the temperature so as to provide a strong stabilizing temperature gradient.
2. Both upflow and downflow machines are sensitive to any horizontal temperature gradients. Such gradients can arise from uneven heating or cooling, heat transfer to or from the electrode chambers, inhomogeneous buffer conductivity due to the sample or selective membrane effects, and electro-osmotic flow which sweeps cooler fluid from the vicinity of the wall to the core of the buffer flow. These gradients cause density variations which deflect the sample stream and cause meandering. Such effects may be diminished by increasing the buffer flow rate or decreasing the gap width, but at the expense of degraded performance.
3. The temperature gradients associated with strong Joule heating combined with cooling through the walls and electro-osmosis produce buoyancy effects which distort the rectilinear axial flow profile, giving rise to secondary flows. Such flows produce unacceptable distortions of the sample stream.
4. Secondary flows driven by electro-osmosis exist near the electrode membranes and at locations where the mobility of the wall material changes abruptly. These flows are slow moving and temperature gradients are large.

Sample trapped in such flows will be difficult to resolve electrophoretically and buoyancy driven restructuring of the main flow will occur in these regions.

5. There is a significant loss of resolution from broadening of the sample stream due to the crescent phenomena. Electric fields arising from conductivity variations or the electrode configuration may drive the sample towards the front and rear walls where electro-osmosis and electrophoresis accentuate broadening due to the crescent phenomenon.

6. With concentrated samples the electrophoretic mobility of particles with thin double-layers (relative to the particle radius) is independent of particle concentration as long as the concentration is below (roughly) 40% by volume. However, the electrical conductivity of the suspension is a strong function of both the double-layer thickness and the volume fraction of particles. For red blood cells at ionic strengths near physiological (0.15M), the conductivity decreases sharply with increasing particle concentration. For particles with thick double-layers the suspension conductivity increases with increasing particle concentration.

Many of the flow disturbances can be dramatically reduced or eliminated by operating the chamber in a micro-g environment. Therefore, devices with a wide gap could be employed at high power levels which would improve resolution.

Electrophoresis experiments aboard the Apollo spacecraft and continuous flow experiments using the McDonnell-Douglas device aboard the Shuttle have established that major difficulties due to buoyancy are suppressed by operating in a micro-g environment. Although the McDonnell-Douglas apparatus is poorly suited for fundamental studies due to geometrical constraints and low power level, the results of experiments carried out by NASA investigators are qualitatively consistent with our findings. In one set of experiments carried out by NASA investigators in the McDonnell-Douglas CFE apparatus it was found that resolution can be degraded in a narrow gap device. Evidently this degradation is due to the combined effects of sample conductivity, electro-osmosis, and shear in the axial velocity profile. Nevertheless, further experimentation is needed to examine the effects of high power levels, chamber geometry, and sample characteristics.

With regard to the electrokinetic properties of suspensions we believe we have enough information to design micro-g experiments on the separation of concentrated suspensions of either polystyrene latices or red blood cells. Techniques have been developed to measure mobility and conductivity with the requisite accuracy and separate effects that contribute to the aggregate properties of the suspension. Given a need to tailor the properties of the buffer to effect the best resolution, however, other experimental and theoretical tools should be

developed. These are needed to probe the nature of the particle surface and relate its properties to the electrophoretic mobility and suspension conductivity. For this purpose AC measurements of the conductivity will be useful since they allow us to decouple events on the surface from processes in the bulk that tend to obscure matters.

RECOMMENDATIONS

It is recommended that a program be initiated to establish theoretically and confirm experimentally the ultimate capabilities of continuous flow electrophoresis chambers operating in an environment essentially free of particle sedimentation and buoyancy. The result will be a definitive evaluation of advantages and disadvantages continuous flow electrophoresis insofar as hydrodynamic issues are involved. This research would focus on hydrodynamic and electrokinetic issues, not the design and operation of a complete electrophoretic separator. Then, with this information in hand one would be able to improve ground-based instruments substantially or design and operate separations devices in a micro-g environment if warranted.

The reasons for this recommendation are as follows. We know that the resolving power of continuous flow electrophoresis for particulate samples can be improved dramatically by operating with a wide gap chamber at high field strengths. Throughput can be increased by using more concentrated samples. Yet neither type of improvement is currently possible in the terrestrial environment due to buoyancy driven motions and sedimentation which interfere with the well-organized rectilinear flow. Two aspects of continuous flow electrophoresis, viz., the behavior of a concentrated sample stream and the extent to which high field strengths (100 V/cm and higher) alter flow structure, must be better understood before the improvements noted above can be realized in ground- or space-based devices.

The specific objective of the micro-g experiments would be to evaluate flows and particle trajectories in a continuous flow electrophoresis chamber to determine if the gains expected by suppressing gravitational effects can, in fact, be realized. The study should have two central themes:

1. Studies of flows in a wide-gap chambers at low flow velocities and high field strengths (high power input and heat loads) to:

(a) Establish the applicability of existing mathematical models of the flow, temperature field, and particle trajectories in a continuous flow electrophoresis chamber to situations where the sample has a low particle number density and where the conductivity mis-match between sample and buffer is negligible.

(b) Ascertain whether or not there are any previously undetected electrokinetic effects that could result in sample stream distortion or meandering. Such effects would have been masked by the more dominant buoyancy driven disturbances encountered at 1-g and would not necessarily appear in the low-field strength studies carried out in the McDonnell-Douglas apparatus.

2. Studies of the effects of particle concentration and the conductivity of the sample and buffer on throughput and resolution. This should involve the use of well-characterized model particles and include ground-based experimental work. Ground-based work would focus on characterization of suspensions with regard to particle mobility and suspension conductivity along with the development of the requisite theoretical models.

Based on the current research we now have enough information to design and interpret experiments for the micro-g environment (and the complementary ground-based counterparts).

INTRODUCTION

Electrophoretic separation techniques have been widely used for the analysis and characterization of biological material. Under terrestrial conditions, electrophoresis and isoelectric focusing are prominent techniques with macromolecules but have been less useful with biological cells or other large particles. Applications of these techniques to preparative scale separations, where significant amounts of material are recovered for subsequent use, are sparse. The major difficulty arises from the effects of sedimentation and buoyancy, effects arising from the action of the gravitational field on density differences of one sort or another. Several techniques have been employed to moderate or eliminate the effect of gravity. These include stabilization of the suspending media by means of a density gradient, rotation of the separation chamber, and minimization of Joule heating effects through the use of special buffers or chamber configurations and pulsed voltages. While such techniques partially mitigate adverse effects, each compromises one or more features of the separation process by altering the surface properties of the particles to be separated or by limiting the size of the sample that can be processed. A detailed discussion of the development of continuous flow electrophoresis dealing with the hydrodynamic and electrokinetic phenomena in a substantial way would be too long to be included here. Instead, a brief description of the salient features is given to place our results in context.

Continuous flow electrophoresis, as developed by K. Hannig ["Die Trägerfreie Kontinuierliche Ablenkungselektrophorese und ihre Anwendung", Zeitschrift für Analytische Chemie, 181, 244 (1961)], A. Strickler ["Continuous Particle Electrophoresis", Separation Science, 2, 335, 1967], and their coworkers, is a technique used for separating small amounts of biological materials. In the process, fluid containing the particles to be separated by electrophoresis moves through a thin chamber under the influence of a pressure gradient. The thickness of the chamber results from a compromise dictated by the need to use a thin chamber to suppress temperature gradients (which cause undesirable flows) and still provide sufficient clearance between the sample and the walls to reduce wall effects. Indeed, in the devices presently available, typical chamber thicknesses vary from 0.5 mm to 3.0 mm. As a result of the small chamber thickness and the attendant necessity for a small sample diameter, current devices do not have sufficient resolving power or sample capacity for some separations, particularly those involving cell populations.

In a thin chamber a flow effect known as the crescent phenomenon serves to spread the sample laterally across the chamber and degrades separation. The crescent formation derives from the effects of shear in both the axial flow (which carries the sample through the chamber) and the electro-osmotic crossflow. Shear in the axial flow gives rise to different transit times and thus different amounts of electrophoretic migration while shear in the crossflow distorts the sample cross-section directly. The overall result is that sample is spread laterally across the chamber which tends to reduce

resolution between different constituents. The crescent phenomenon can be minimized by making the sample cross-section small compared to the chamber thickness. In an already thin chamber this leads to smaller throughput. Lateral spreading of the sample is further accentuated by the effects of non-uniform electric fields in the neighborhood of the sample. Such fields can carry sample towards the front and rear walls of the chamber where electro-osmosis carries the sample laterally.

Heretofore apparatus has been designed by essentially empirical methods and attempts to increase yield, resolution, and reliability of the continuous flow device have failed due in large part to an incomplete understanding of the basic processes involved.

Two lines of attack offer ways of circumventing the problems mentioned earlier and thereby improving resolution and throughput. One is to increase the chamber thickness so as to allow a larger sample cross-section which, at the same time, spans only a small fraction of the chamber. This would allow for increased sample throughput, which is proportional to the sample area, but retain resolution by keeping the ratio of the sample diameter to chamber thickness small.

However, flow structure in a thick chamber for both downflow (the flow direction commonly employed) or upflow at 1-g is unsuitable at 1-g. The sample tends to meander or large scale recirculations are present which thwart separation. The reasons for this sort of flow behavior were not understood when this research was initiated.

The other line of attack is to increase the particle concentration in the sample. Increasing the sample concentration would increase throughput but not resolution. At 1-g sample sedimentation causes unacceptable mixing but in a micro-g environment sedimentation would be suppressed. Nevertheless, there are considerable effects on the sample conductivity and these can cause undesirable spreading of the sample. The extent to which particle mobility and sample conductivity are effected by particle concentration were unknown at the initiation of the research.

Because of the lack of fundamental understanding of the processes noted above an extensive investigation of continuous flow electrophoresis and related electrokinetic phenomena was carried out at Princeton under the auspices of NASA's Microgravity Sciences and Applications Program. Complementary work at MSFC under the direction of Dr. R. S. Snyder is consistent with the results found at Princeton. As a result our understanding of the basic processes has advanced to a point where a definitive set of experiments can be designed to establish the resolving power and capacity of continuous flow devices. The scope and principal results of the Princeton investigations are summarized below.

STRUCTURE OF FLOW IN A CONTINUOUS FLOW ELECTROPHORESIS CHAMBER (CFE)

1. Purpose

The aim of this part of the investigation was to describe the significant features of the axial (pressure gradient driven) flow in the presence of buoyancy forces. Since it was known that many of the deleterious effects of buoyancy could be avoided by using narrow gap devices of the sort developed by Hannig and Strickler, emphasis was placed on developing an understanding of: (i) the sort of processes that "destabilize" this flow and (ii) the flow structure in a channel with a wide gap operated at high power levels, the sort of system that could fractionate significant amounts of material. We sought, in essence, to understand which hydrodynamic processes caused the flow to "meander" in the device as the power level or the gap thickness was increased and then to establish the flow structure that would prevail if the "meandering" could be avoided.

2. Results

It was established theoretically that flows of the sort encountered in CFE chambers are very sensitive to hydrodynamic instabilities due to axial temperature gradients(3)*. The critical Rayleigh numbers are roughly three orders of magnitude less than those for fluid layers that are bounded above and below. It was also shown, using a Hele-Shaw model of the flow(7), that very small thermal inhomogeneities (a fraction of a Kelvin over an area of a few square millimeters) would cause a substantial flow reversal. This was verified in experiments at MSFC(33). This extraordinary sensitivity to small thermal excursions is believed to be responsible for much of the "meandering" observed when the gap thickness or the power level are increased. Presumably problems of this sort can be overcome by careful design of the heat transfer system so as to provide a strong stabilizing temperature gradient. Given that this is possible, the task is to investigate the structure of the flow that would ensue in a wide gap configuration with a high field strength, configured so as to suppress the instability identified above.

Several sorts of mathematical models were constructed to describe situations that would prevail in a wide gap device operated at high power inputs. To establish how much of the flow channel is occupied in setting up the temperature field, a thermal entrance length calculation was carried out for a rectangular channel with internal heat generation in the flow and heat transfer to the four bounding walls (4). Calculations of this sort are a necessary part of the thermal design mentioned above.

Because the axial flow is driven by pressure gradients and buoyancy forces, it is possible to reverse the flow if the

* Astrisks refer to publications emanating from the work and are listed in the bibliography.

buoyancy effect is large. Calculations of the flow structure showed that reversals are inevitable, irrespective of whether the main flow is upward or downward as long as the power input is large. Two dimensional calculations were done with a constant fluid properties model(2) as well as one dimensional calculations with a variable properties model(1). The latter calculation showed that effects due to the lower viscosity in the core of the channel exacerbated the flow reversals due to buoyancy. These calculations serve to set limits on the operation of the CFE device at 1-g since flow reversals cause unacceptable mixing in a device designed for electrophoretic separations.

The next step in our program was to take account of more realistic electrode membrane configurations(6,7,17,19). Because the membranes that isolate the electrodes from the chamber may be arranged in a variety of configurations it is necessary to establish the merits of each. The importance of allowing for realistic configurations can be appreciated once it is recognized that the structure of the electro-osmotic crossflow is in large measure set by the electrode arrangement. Furthermore the electro-osmotic flow affects the temperature field which may dictate the structure of the axial flow. An additional complication arises because the electro-osmotic characteristics of the membranes differ from those of the materials that make up the remainder of the inner surfaces of a CFE chamber. Accordingly, a general model of the three dimensional flow structure was developed which enables us to calculate the axial development of the electro-osmotic crossflow and the restructuring of the axial flow. This includes: (i) the effects of electrode configuration on the structure of the electric field and the flow, (ii) the electrokinetic properties of the electrodes and the other surfaces that bound the flow, and (iii) the effects of buoyancy(17,19).

Another result of our mathematical modelling effort was to construct a general simulation of the fractionation of particle mixtures in a CFE device(14). The computer program is flexible so that the electrophoretic mobility distribution in the sample can be specified at will; flow rate, sample size, field strength, buffer properties, and electrode length are treated in the same fashion. The variation of fluid and particle properties with temperature and the effect of buoyancy on the axial flow are also taken into account. With this program we can analyse and compare various combinations of buffers, field strengths, and flow rates to optimize a particular separation.

ELECTROPHORETIC MOBILITY OF INDIVIDUAL PARTICLES AND THE ELECTRICAL CONDUCTIVITY OF SUSPENSIONS

1. Purpose

The second major focus of our work was the electrokinetic properties of suspended particles(8-13,15,16). Studies were made of: (i) the electrophoretic mobility of suspended particles in dilute and concentrated systems and (ii) the electrical conductivity of dilute and concentrated suspensions. The sedimentation potential was also investigated in connection with the latter study(5). In these investigations we sought to establish: (i) how the mobility of the suspended particles is related to the buffer composition and the presence of nearby particles, and (ii) how the bulk conductivity of a suspension is related to the buffer properties, the electrokinetic properties of the suspended particles, and the number of particles per unit volume.

Although the reasons for studying the mobility of individual particles are obvious, those behind the studies of conductivity may not be as clear. They are no less compelling, however. In other electrophoretic separation processes, isotachophoresis and isoelectric focusing of soluble materials, for example, it has long been known that the sample conductivity affects separation rates by altering the local electric field. Analogous effects are present in free fluid electrophoresis of particulate suspensions. Here the fact that the conductivity varies from point to point in the neighborhood of the sample stream engenders a non-uniform electric field. Components of this field drive sample constituents towards one of the walls where electro-osmotic spreading degrades the separation. Thus it is important to know how the conductivity is related to the properties of the sample. The results of the several parts of our study are summarized below.

2. Results

Measurements of the electrophoretic mobility of individual particles and the electrical conductivity of dilute suspensions showed that the familiar zeta-potential does not suffice to fully characterize the electrokinetic properties of the surface of a particle. This is due to transport processes in the Stern layer(9). A model was developed to characterize the influence of these transport processes(11,12). The results of calculations made with the model show that much of the surface charge can be mobile, and that the amount of charge depends on the buffer composition. The model could be used, along with experimental data, to optimize buffer composition so as to accentuate intrinsic differences between the mobilities of different subpopulations of suspended particles.

Measurements of the bulk conductivities of more concentrated suspensions disclosed how the conductivity varies with the volume fraction of particles. Correlations and a simplified theory were developed to analyze the results(16). It was demonstrated how the counterions from the particle charging process play a major role in determining the bulk

conductivity. These contributions must be taken into account in adjusting compositions so as to optimize the sample conductivity-buffer conductivity combination.

The results mentioned thus far were obtained using polystyrene latex particles. Since these particles are opaque it is difficult, if not impossible, to measure electrophoretic mobilities directly in concentrated systems. We modified some of the existing techniques for making red blood cell ghosts so as to prepare transparent suspensions. Then by using tracer particles (red blood cells prepared so as to have the same electrokinetic properties as the ghosts) we could measure particle mobilities directly. This was done with homogeneous suspensions (where the ghosts and the tracers all have the same mobility) and with heterogeneous suspensions (where the ghost population is a mixture of two subpopulations with different mobilities). We found that in systems with thin double layers (i.e. near physiological ionic strength) the particle mobility was the same as the intrinsic mobility (i.e. that measured at infinite dilution) irrespective of the volume fraction of particles in the suspension for a homogeneous buffer(13,16). This is a very important result since it implies that separation in a continuous flow device will not be degraded with concentrated samples. We also measured the conductivity of the suspensions and developed correlations for the effect of particle volume fraction on the bulk conductivity(18).

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